HAYATI
Journal of Biosciences

Vol. 26 No. 2, April 2019 81-89
DOI:10.4308/hjb.26.2.81
ISSN: 1978-3019
EISSN: 2086-4094

In vitro and *In vivo* Antiplasmodial of Stem Bark Extract of *Garcinia husor*

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ARTICLE INFO

Article history:
Received July 18, 2018
Received in revised form September 18, 2018
Accepted February 1, 2019

KEYWORDS:
Antiplasmodial,
Garcinia,
parasitemia,
P. berghei,
P. palcifarum

ABSTRACT

Garcinia husor **is one of the folk medicines in Maluku-Indonesia. This species has been used for the treatmet of Malaria disease. The phytochemical contents and antiplasmodial activity not reported yet. In this study we evaluated the quantitative phytochemicals, *in vitro* and *in vivo* antiplasmodial activity of stem bark ethyl acetate extract. *In vitro* assay was done using *P. falciparum* 3D7 strain sensitive of chloroquine. For *in vivo* analysis, four groups of *M. musculus* were infected by *P. berghei* and their parasitemia levels were for 7 days of treatment with ethyl acetate extract; hematological and biochemical parameter were analyzed at the end of experiment. The result showed ethyl acetate extract with the TPC (169.47 mg GAE/100 g ±0.61) and TPC (167.37 mg QE/100 g ±1.05) was active against *P. falciparum* 3D7 strain (IC_{50} value of 0.31±0.43 µg/ml). The animal treated with extract showed suppression of parasitemia to 87.57±1.41% compared with the *P. berghei* infected-mice (negative control), ED_{50} value of 22.30 mg/kg BW. The dose of extract in 200 mg/kg BW was reduce parasitemia of infected mice with *P. berghei* more potential. The ethyl acetate of the stem bark *G. husor* with has antiplasmodial properties and future investigation are necessary to elucidate its mechanism of action.**

1. Introduction

Malaria is a major infectious disease with a high mortality in the developing country (Boampong *et al.* 2015). Until now, efforts have been made to treat this disease related to the development of alternative antimalarial, due to the emergence of drug-resistant strain. Many plants are used therapeutically in alternative and traditional medicine to treat infectious diseases. New and safe antimalarial drugs are urgently needed and natural products can represent a vast source of leading molecules (Oliveira *et al.* 2014).

Garcinia species are known to contain phenolic compounds such as xanthones, benzophenones, flavones, and depsidone (Nilar *et al.* 2005). Phenolic compounds showed that a wide range of biological and pharmacological properties, e.g. antioxidant, anti-inflammatory, antimicrobial, antiplasmodial, and cytotoxic activities (Minami *et al.* 1994; Vlientinck *et al.* 1998; Merza *et al.* 2006; Cos *et al.* 2008; Chin *et al.* 2008; Elfita *et al.* 2009). Benzophenones have been reported as anti-HIV, trypanocidal and cytotoxic agents (Gustafson *et al.* 1992; Vlietinck *et al.* 1998; Williams *et al.* 2003; Merza *et al.* 2006; Cos *et al.* 2008).

Garcinia husor (locally named "manggustan hutan" in Indonesia) is a medium sized tree ocurring in Maluku. In some Maluku communities, the stem bark of *G. husor* used in the treatment of malaria as endemic area but this has not been scientifically verified. This study was designed to evaluate the antimalarial potential of the ethyl acetate extract stem bark *G. husor* using *Plasmodium falcifarum* D37 strain *in vitro* and *in vivo* at *P. berghei* infected mice model.

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2. Materials and Methods

2.1. Plant Material

The stem bark of *G. husor* was collected from Allane Forest at Ambon island, Indonesia. This plant was identified and a voucher specimen (No. 52) was deposited at Biology Laboratory of Biology Departement, Pattimura University, Indonesia.

2.2. Preparation of Extract

The stem bark of *G. husor* were dried at room temperature and reduced to coarse powder using a disk mill SMJMA, FFC-15, Shandong Jimo. Dried stem bark powder (500 g) was mixed ethyl acetate and filtered. The solvent was evaporated under reduce pressure (Rotavapor R-210, Buchi, Switzerland) to obtain a solid mass extract of ethyl acetate (13.3% w/w).

2.3. Screening for Secondary Metabolites

The ethyl acetate extract was screened for the presence of phytochemicals using standard procedures described elsewhere (Njoku and Obi 2009; Khan *et al.* 2011; Tiwari *et al.* 2011).

2.4. Determination of TPC and TFC

Total phenolic and flavonoid contents were determined by Folin-Ciocalteu's and aluminium chloride colometric methods, respectively (Singh *et al.* 2013) following quantification on the basis of standard curve of gallic acid (GAE) and quercetin (QE) equivalent, respectively.

2.5. Antimalarial Assays

2.5.1. *In Vitro* Test

The antimalarial activity of the extract was determined by the procedure described by Widyawaruyanti *et al.* 2007. In brief, extract dissolved in DMSO (10^{-2} mol L^{-1}) and kept at -20°C until used. The malarial parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plate in the presence of a wide range of concentrations of each compound. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa stain.

% growth = % paristemia - DO
% inhibition = 100% - ((Xu/Xk) x 100%)

Where:
DO :% growth at 0 o'clock
Xu :% growth at solution test
Xk :% growth at negative control

Based on percent inhibition data, an analysis was conducted between the concentration of the test and the percent inhibition using probit log analysis to determine the IC_{50} value or concentration of the extract which could inhibit parasite growth by 50%.

2.5.2. *In Vivo* Tests

Animals and husbandry *M. musculus* weighing 20-24 g were obtained from Biology Laboratory Pattimura University, Indonesia. The animal were housed in stainless steel cages (34 x 47 x 18 cm) with soft wood shaving as bedding, fed with normal commercial pellet diet given water *ad libitum* and maintaned under ambient laboratory conditions. All procedures and techniques used in these studies were in accordance with the National Institute of health Guidelines for the Care and Use of Laboratory Animals (NRC 1996). All ethical protocols used for the study were approved by the Departemental Ethics Committe.

2.5.2.1. Animals

Four-week-old *M. Musculus* from the Zoology Laboratory of Pattimura University were used in the experiments. Prior to the experimental produres, the animals were kept at 22±2°C on a 12 h dark-light, with balanced feed and water *ad libitum* for 15 days. All experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care adopted by the Animal Use Ethics committe of Airlangga University.

2.5.2.2. Acute Toxicity Evaluation

The acute toxicity assay was performed following the guidelines of The Organization for Economic Cooperation and Development (OCDE)-423/2001. When previous studies have assessed the acute toxicity of a known sample, the iniatial dose used can be the maximum dose without side effects. *G. husor* was tested in three groups of three animals each that were analyzed every 30 min during the first 4 h, periodically for 24 h and daily until the 14th day. Several parameters such as general activity, irritability, contortion, ataxia, tremors, convulsion, piloerection, hypothermia, breathing, cyanosis, hyperemia, and death were analyzed.

2.5.2.3. Induction of the Experimental Malaria Model

The malaria model was induced using the *P. berghei* ANKA (PbA) line. For the inoculation of the experimental groups, a blood drop from infected animals was collected from the terminal portion

of the tail to analyze parasite density. The blood content of animals with a parasite density higher than 10% in the ascent phase was collected by cardiac puncture using an insulin syringe washed with the anticoagulant agent EDTA. To guarantee uniform parasitemia in the different experimental groups, animals were infected intraperitoneally with a standardized inoculum of approximately 10^6 parasitized erythrocytes in 100 µl PBS.

2.5.2.4. Determination of Parasite Desity and Parasitemic Suppression

Parasites were investigated in mouse tail blood smears fixed in absolute methanol for 2 min and stained with 10% Giemsa for 15 min. Percent parasitemia was determined in each animal as the ratio between the quantity of parasitized erythrocytes and the total quantity of cells (500 cells per smear):

Parasitemia was determined on the 4th days

$$\% \text{ parasitemia} = \frac{\text{quantity of parasitized erythrocytes}}{500}$$

post-inoculation of BALB/c mice with PbA line. The model proposed by Girma *et al.* (2015) was used to determine the suppression potential of oleoresin against the parasitemia rate of *P. berghei*-infected mice.

2.5.2.5. Experimental Design

Mice were randomly divided into four groups of 3 animals each: Group 1 (G1), untreated *P. berghei*-infected mice; Groups G2, G3, and G4, *P. berghei*-infected mice treated orally with ethyl acetate *G. husor* stem bark extract, 10, 100, and 200 mg/kg/day, respectively. Extract was suspended with 5% CMC-Na as vehicle. All treatments began on the 4th day after *P. berghei* inoculation, when parasite density was first determined to confirm infection. For the collection biological material at the end of treatment, animals were kept on their habitual diet without fasting due to the fragility of the malarial state.

2.5.2.6. Statistical Analysis

Data were analyzed statistically with Microsoft Excel 2010 and SPSS v22. Result were analyzed by ANOVA followed by LSD if significant difference. p value <0.05 was considered statistically significant. All data were expressed as mean ± SD (triplicate measurement).

3. Results

3.1. Preliminari Phytochemical of Ethyl Acetate Extract

The qualitative and quantative phytochemical of ethyl acetate extract was prepared from *G. husor* stem bark. The results of preliminary tests of different phytochemicals extract revealed the presence of alkaloid, terpenoids, flavonoid, tannins, and phenolics (Table 1).

The quantitative of phytochemical by determined of TPC (Total phenolic content) and TFC (Total flavonoid content) from extracts. The amount of total phenolic compounds present in extract was determined from linear regression equation of calibrate curve, [y= 0.0045x + 0.0481 (R2=0.9581)] and expressed as Gallic acid equivalent in mg/ml of extract. Table 1 depicts the result of assesment of total phenolic content in the tested extracts. It is found that, stem bark of *G. husor* ($p<0.05$) displayed high contents of total phenolics in aquoeus extracts showed 169.47 mg GAE/100 g ±0.61 mg GAE/100 g.

The total flavonoid in extract was determined from linear regression equation of calibration curve obtained from the different concentration of quercetin [y=0.009x + 0.0161 (R2=0.983)] and the results was expressed as Gallic acid equivalent in mg/ml of extract (Table 1). However, extract demonstrated considerably high amount of flavonoids in stem bark *G. husor* with 167.37 mg QE/100 g ±1.05 (Table 1).

Table 1. Qualitative and quantitative phytochemical of ethyl acetate of *G. husor* stem bark extract

Secondary metabolites tested	Ethyl acetate extract
Qualitative of phytochemical	
Alkaloids	+
Terpenoids	+
Flavonoids	+
Saponin	-
Phenolics	+
Tannins	+
Quatitative of phytochemical	
Total phenolic content	169.47 mg GAE/100 g ±0.61[a]
Total flavonoid content	167.37 mg QE/100 g ±1.05[b]

+:present, -:undetected
[a] Results are expressed as gallic acid equivalents//100 g of extract
[b] Results are expressed as quercetin equivalents//100 g of extract
Value were performed in triplicated and represented as mean ± SD. Level of significance: ($p<0.001$)

3.2. *In Vitro* Antimalarial Activity

The *in vitro* assay, antimalarial activity with the treatment of of *G. husor* stem bark ethyl acetate extract showed a decrease in parasite percentage of growth according to increased concentration, 0.01 µg/ml (3.28%); 0.1 µg/ml (2.54%); 1 µg/ml (1.74%); 10 µg/ml (1.01%); 100 µg/ml (0.36%) (Figure 1a). Thus, the inhibition of parasite growth increased as the concentration increased, 0.01 µg/ml (22.89%); 0.1 µg/ml (40.25%); 1 µg/ml (59.04%); 10 µg/ml (76.29%); 100 µg/ml (91.55%) (Figure 1b). Chloroquine as a positive control showed 100% inhibition at 100 µg/ml concentration and the lowest inhibition of 59.09%, this is equivalent to inhibition of the extract at 1 µg/ml of concentration. Extract exhibiting IC_{50} value ≤10 µg/ml was cosidered to be active. Those exhibiting IC_{50} value in the range 10 to ≤10 µg/ml were considered moderately active. Extract exhibiting IC_{50} value ≥10 µg/ml was considered in active (Bagavan *et al.* 2011; Bunyong *et al.* 2014; Lima *et al.* 2015). Extract ethyl acetate potent inhibitory activity, with an IC_{50} value of 0.31 µg/ml less than that antimalarial drug, chloroquine (IC_{50}, 0.006 µg/ml). The inhibitory activity of these extract supports the traditional use of the dried stem bark of *G. husor* as an antimalarial drug.

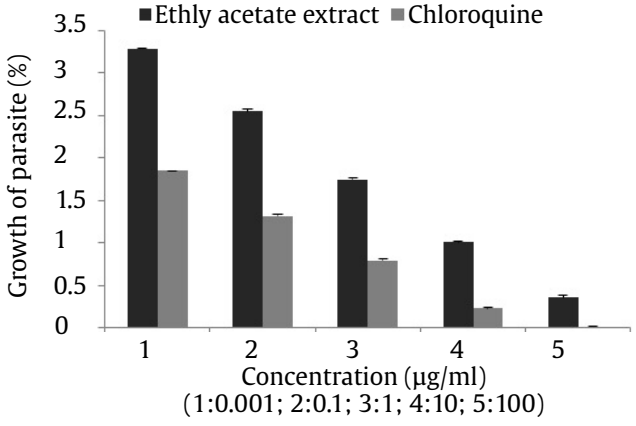


Figure 1. (a) Growth (%) and (b) Inhibition (%) of *P. falcifarum* incubated 48 hours with *G. husor* stem bark ethyl acetate extract. Value were performed in triplicated and represented as mean ± SD. Level of significance: (p<0.001)

3.3. *In Vivo* Antimalarial Activity

3.3.1. Acute Toxicity Evaluation

The administration of the maximum dose of ethyl acetate *G. husor* stem bark extract did not promote any changes such as loss of the hair or color, tremors, increased salivation, diarrheae, lethargy, increased or reduced sleep, pain or suffering, or weight loss.

Body weight is one of indicator that shows the state of malaria disease. Generally, in the *P. berghei*-infected state, the body weight of the mice will decrease as the number of infected red blood cells increases. Thus, by observing the weight changes of the mice, it is hoped to know the relationship with the change of parasitemia number in mice. In Table 2, *P. berghei* infection caused significant($p<0.05$) decrease in the body weight-gain in the mice relative normal of all groups. All tested doses of the aquoeus extract significantly protected the mice from body weight loss. Thus it can be said that the extract is safe and effective as an antimalarial and does not significantly influence body weight loss of mice (Fentahun *et al.* 2017).

3.3.2. Quantitative Analysis of the Evaluation of Parasitemia

Quantitative analysis of number of parasites in blood samples collected on 4th day post-inocultion showed level growth of parasite in the mean presentage of G1, infected only (48.3±0.007%) higher than G2 (27.66±0.06%), G3 (21.66±0.01%), G4

Table 2. Effect of extract on the mean body weight in *P. berghei* infected mice

Treatment	Body weight (g)		Changes in body weight (g)
	Initial	Final	
Infected only (G1)	20.17±0.05	19.84±0.11	-0.32±0.06
Infected + extract dose10 mg/kg BW (G2)	20.58±0.39	20.42±0.47	-0.16±0.10
Infected + extract dose 100 mg/kg BW (G3)	21.25±0.12	21.20±0.11	-0.05±0.02
Infected + ekstract dose 200 mg/kg BW(G4)	21.93±0.29	21.92±0.36	-0.01±0.01

Values are given as mean ± SD (n=3)
*Significantly different from normal (p<0.05)

(6.0±0.01%) (Figure 2). Effects of extract on *P. berghei* infected *M. musculus*. The results showed that an average percentage of inhibition against *P. berghei* of the infected only group (G1) and the infected treatment extract group (G2, G3, and G4) where the increased of dose treatment decrease percent growth of parasite. This indicates higher percentage of inhibition G4 (87.37%) than G2 and G3. The G1 as negative control showed that higher growth of parasite (48.30±8.17%) and can not inhibit of parasite. So it can be said that ethyl acetate *G. husor* stem bark extract has good antimalarial activity because at a dose of 200 mg/kg BW showed percentage of inhibition ≥50% (Munoz *et al.* 2000).

Extract with persentage of inhibition ≥50% an *in vivo* assay at a dose of 100 mg/kg BW was classified as having excellent anti *Plasmodium* activity (Bantie *et al.* 2014; De Villiers *et al.* 2017). Based on this classification, the ethyl acetate *G. husor* stem bark extract have exelent antiplasmodial because at a dose of 100 mg/kg BW has been able to inhibit the growth parasite of *P. berghei* ≥50%. Furthermore, probit analysis obtained ED_{50} value ethyl acetate *G. husor* stem bark extract of 22, 30 mg/kg BW.

The results of change level parasitemia by ANOVA test and futher analiysis LSD showed that a significant difference in the mean presentage of decrease of parasitemia, $p<0.05$ G1 (0.01±0.07%), G2 (0.10±0.06%), G3 (0.24 ± 0.01), G4 (0.34±0.01%). The result showed that parasitemia was reduced in all treated groups in relation to untreated *P. berghei*-infected mice (G1) (Figure 3). The infected untreat extract (G1) was significantly different with the doses of 10 (G2), 100 (G3), and 200 mg/kg BW (G4), the dose 10 mg/kg BW (G2) was not significantly different with the dose of 100 mg/kg BW (G3), but the dose of 100 mg/kg BW (G3) was significantly different with a dose of 200 mg/kg BW (G4).

Malaria infection also causes renal damage due to high parasitemia, when parasitized erythrocytes become hard, with reduced deformability and changes conformation. This increases blood viscosity, slowing and microcirculation and altering renal blood flow. Additionally, the cytoadherence of infected erythrocytes alters the local circulation, aggravating the renal injury. Our result showed that treatment with extract may contribute to an increase in the microcirculation, reducing erythrocyte cytoadhence, with a significant decrease of parasitemia.

The level of parasitemia is obtained from thin blood preparation by counting the number of infected cells *P. berghei* (ring-shaped trofozoid, advanced stage trofozoid, and schizon) in 1000 erythrocytes. Erythrocytes containing old trofozoites and schizons have obviously coarse points (Maurer's point) spread over two-thirds of the erythrocytes. The more trophozoites and schizons, the higher the level of parasitemia (Figure 4).

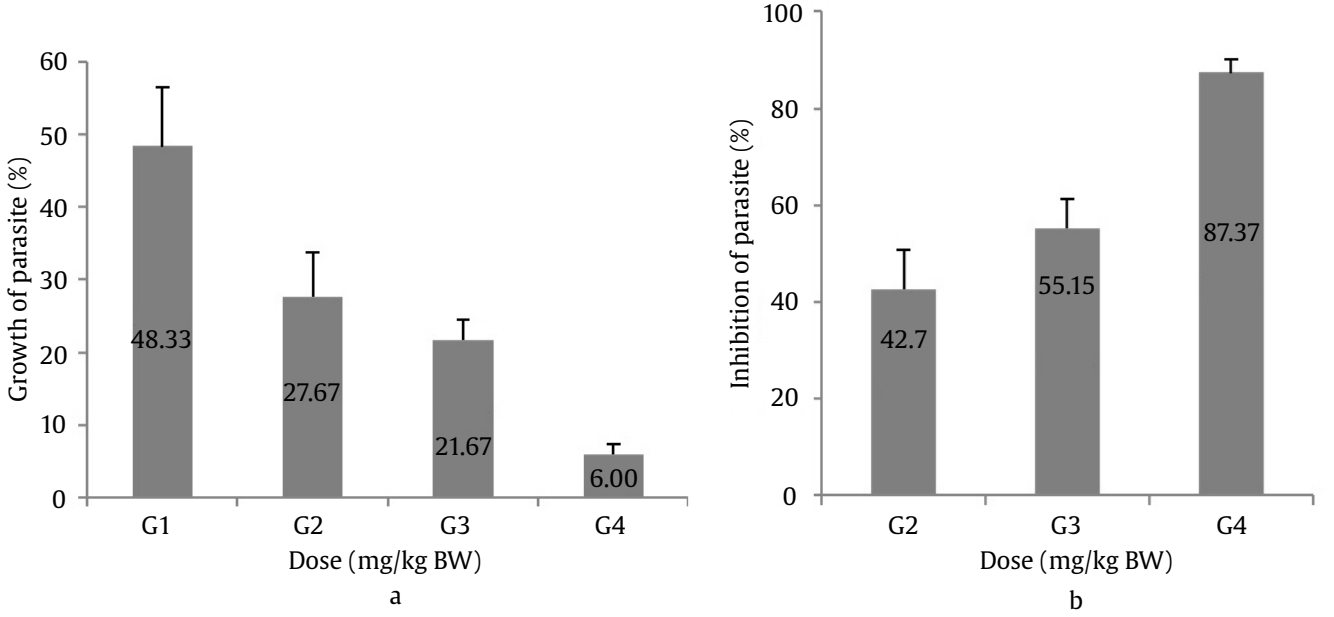


Figure 2. (a) Growth of parasite (%) and (b) inhibition parasite (%) in *M. musculus*. Each bar represents the mean ± SD level parasitemia of animal belonging to G1 (of *P. berghei* infected-mice), G2 to G4 (aquoeus *G. husor* stem bark extract 10, 100, and 200 kg/BW), respectively). Significantly different ($p<0.001$) in level parasitemia of G1 compared to G2-G4

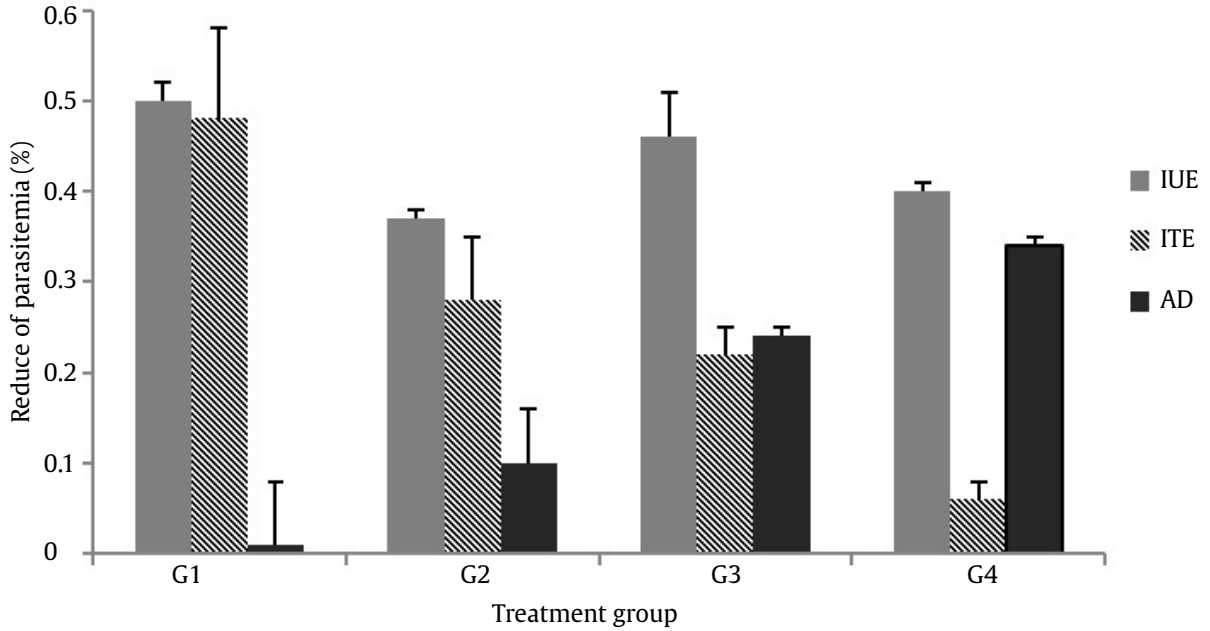
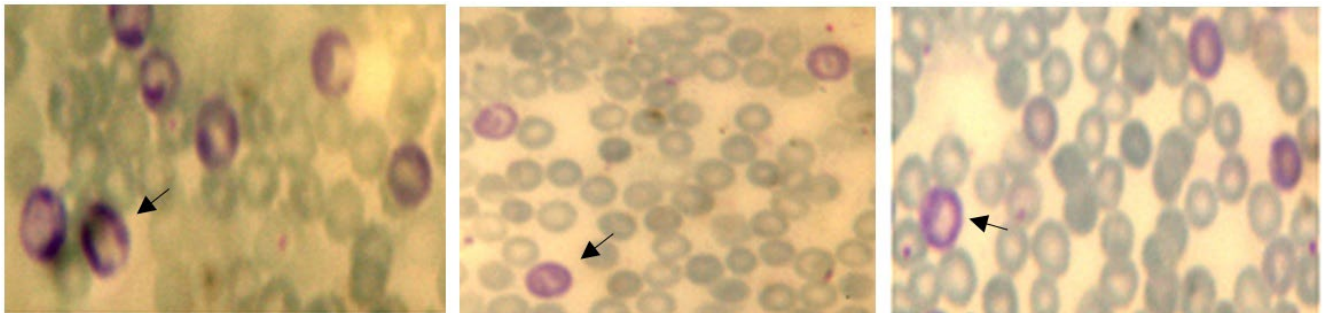


Figure 3. Reduce of parasitemia (%). Each bar represents the mean ± SD reduce parasitemia of animal belonging to G1 (of *P. berghei* infected-mice), G2 to G4 (ethyl acetate *G. husor* extract 10, 100, and 200 kg/BW), respectively). IUE: infected untreated of extract; ITE: Infected treatment extract, AD: average of difference. Significantly different ($p<0.05$) inreduce of parasitemia of G1 compared to G2-G4



a b c

Figure 4. Microscopic evidence of erythrocytic is infected with *P. berghei* after treatment antimalarial activity of *G. husor* extract. Infected erythrocytes (trofozoite ring form) are indicated by arrows. (a) dose 10 mg/kg BW, (b) dose 100 mg/BW, (c) Dose 200 mg/BW

4. Discussion

During the prospection of new subtances with antimalarial potential an important feature is required: the subtances must present reduced IC_{50} value assays, minimizing the lack of drug specifity and side effect (de Souza *et al.* 2017). Extract have IC_{50} value ≤ to *P. falcifarum* can be continued to isolation of active compounds in medicinal plant (Oliveira *et al.* 2014). Ethyl acetate *G. husor* stem bark extract showed reduced IC_{50} value (0.31 µg/ml) against *P. falcifarum* 3D7 strain. Moreover, it is expected that a potent antimalarial agent (Katusno *et al.* 2015) and be continue *in vivo* antimalaria assay.

The present results shown a low toxicity of ethyl acetate extract even at doses higher than used in the study. The absence of clinical features of toxicity or weight variation is important, mainly because in the malaria state the animal can present symptoms belonging to both states such as pelage change, lethargy and weight loss which could interfere which the results.

The observation that no death caused by an oral highest dose of 200 mg/kg body weight of ethyl acetate extract of the *G. husor* stem bark could imply the safety of the plant to be used in the treatment of Malaria as also suggested in Akele *et al.* 2013 and Murithi *et al.* 2014. The acute toxicity result of the present study suggested that

the oral extract at the highest dose (200 mg/kg BW) and included in the category of practically non-toxic (OECD 2008). The experimental determination of acute toxicity at the extract dose of up to 2000 mg/kg body weight of mice may justify the use of this plant for malaria treatment.

In vivo antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a respective percent parasite suppression equal to or greater 50% at doses of 500, 250, and 100 mg/kg body weight per day (Munoz *et al.* 2000; Deharo *et al.* 2001). Based on this classification, the extract of *G. husor* are considered to exhibit good antiplasmodial activity, with dose dependent inhibition against *P. berghei* infection in mice.

Malaria is an inflammatory cytokine-driven disease that often results in mortality due to sequestered parasitized red cells preventing sufficient oxygen access to where it is needed together with mitochondria inability to generate enough adenosine triphosphate to maintain normal cellular function (Clarck *et al.* 2010). *P. berghei* parasite is used in predicting treatment outcomes of any suspected antimalarial agent due to its high sensitivity to chloroquine, making it the appropriate parasite for this study (Boampong *et al.* 2015). In this study, three doses of the ethyl acetate stem bark extracts of *G. husor* exerted curative antimalarial activities against the blood stage of *P. berghei*. Ethyl acetate extract give high effect for reduce level of parasitemia in dose 100 mg/kgBW 24±0.01% and 87.37±1.41% inhibition of parasite of *P. berghey*–infected mice. This result showed that effect ethyl acetate extract lower than treated orally dose 100 mg/kg BW with artemisinin were 54±0.14%; 95%, respectivelly (de Souza *et al.* 2017). This implies that the active principles for the observed antimalarial effects are present in ethyl acetate extract. It is proposed that the presence of alkaloids, triterpenoids, flavonoids, phenolics, and tannins could be responsible for the observed curative antiplasmodial activities of the extract but further experiments are needed to confirm this hypothesis. Some alkaloids that act against malaria parasites inhibit protein synthesis by intercalating with DNA of the parasite (Okokon *et al.* 2008; Christian *et al.* 2012). In addition, flavonoids and tannins have been suggested to act as primary antioxidants or free radical scavengers that can counteract the oxidative damage induced by the malaria parasite (Gautam *et al.* 2011; Kayani *et al.* 2016). Numerous studies have implicated oxidative stress in several pathological conditions such as stroke, diabetes, Parkinson's disease and so on. Apart from their detrimental effects, free radicals are also involved in cellular signaling and as carriers for iron requirement needed by parasites for survival in a host. Iron is a crucial nutrient for survival and replication of microorganisms such as *Plasmodium* parasites. Thus, the ability of an antioxidant to chelate/sequester iron needed by the *Plasmodium* parasite will adversely affect the survival of the parasite in a host. Flavonoids in particular have the ability to chelate iron and they have been suggested that such compounds with the ability to sequester iron should be employed in treatment of infections (Chandrashekaran *et al.* 2010). Ethyl acetate extract have high total flavonoid content, which possibly contributed to the antiplasmodial effect observed in the mice. Some terpenoids attack the broadest age range of parasites, from the tiniest rings that have recently invaded erythrocytes to more mature stages of parasites such as developing trophozoites and schizonts. Their relatively broad stage-specificity of action has been reported to extend to the ability to impede development of gametocytesm (Yoo *et al.* 2008; Coma-cros *et al*. 2018). The data provide evidence that stem bark extracts of *G. husor* antiplasmodial activity which may partly be mediated by its phytochemical compounds.

In the qualitative and quantitative analysis of this plant studied, some of these secondary metabolites were present and saponin was absent. It has been reported that several plant constituents, vis; flavonoid, tannins, xanthone, phenolic, and triterpenoid posses protein-binding and enzim-inhibiting properties (Selvanayagam and Ghanavendhan 1995; Fale *et al.* 2012; Oluwatosin *et al.* 2014). The likely mechanism of action of this extract contain flavanoid may be inhibition of key pathognic enzymes of the parasite since extract is known to interference with enzymes sistem (Okunji *et al.* 2007; Penduka *et al.* 2011). Alkaloids are important phytochemicals, that are said to be pharmacologically active. Quinine, an alkaloid, is popular for is antimalaril activity against the malaria parasite (Bankole *et al.* 2016). Triterpen, 3b-hydroxy-23-oxo-9,16-lanostadien-26-oic acid or garcihombronane D from *G. celebica* showed a selective activity against *P. falcifarum* (Elfita *et al.* 2009).

Pearson's correlation coefficient (r) showed high and positive correlation between phenolic content in extract with antimalarial activity (Table 3). In the

Table 3. Pearson's correlation coefficient (r) of TPC, TFC ethyl acetate *G. husor* stem bark extract and antimalarial activity

	TPC	TFC	APf	APb
TPC	1*			
TFC	0.888*	1*		
APf	0.904*	0.999*	1*	
APb	0.747*	0.968*	0.959*	1*

present study, TPC and TFC to be analytical markers since they were the major components of the test sample. Similarly, phenolic were have confirmed its antimalarial activity. On the other hand, due to the complexity of the sample, it is difficult to state that molecule is responsible for the antimalarial potential of extract, with a synergistic effect probably playing an important role in the activity.

The previous studies reported that phenolic components from *Garcinia* genus have antimalarial potential i.e. α and β-mangostin (*G. mangostana*) 1,5-dihidroksi-3,6-dimetoksi-2,7-diprenilsanton isosantoximol (*G. griffithii*), kolaviron (*G. cola*) (Mahabusarakham *et al.* 2006; Oluwatusin *et al.* 2014).

Malaria infection also causes renal damage due to high parasitemia, when parasitized erythrocytes become hard, with reduced deformability and changes conformation. This increases blood viscosity, slowing and microcirculation and altering renal blood flow. Additionally, the cytoadherence of infected erythrocytes alters the local circulation, aggravating the renal injury. Our result showed that treatment with extract may contribute to an increase in the microcirculation, reducing erythrocyte cytoadhence, with a significant decrease of parasitemia.

The present study revealed that ethyl acetate stem bark *G. husor* extract effective herb with significant antiplasmodium i*n vitro* test antiplasmodium against *P. falcifarum* 3D7 strain and *in vivo* test in *M. musculus* infected *P. berghei* (ANKA) strain. The biological activities observed in the study could be attributed to the higher phenolic and flavonoid contents of the extract. The result obtained in this study demosntrated that the ethyl acetate of *G. husor* stem bark extract potent antimalaria activity. However, this appears to the first report of antimalarial properties of stem bark *G. husor* extract *in vitro* and *in vivo* research. The result of this study suport the traditional claim of the plant in the use for malaria treatment, and effort are currently underway to identify the bioactive components of the plant and toxicological effects in animals.

The overall, result showed that ethyl acetate of *G. husor* stem bark *in vitro* and *in vivo* has good activities as antipalsmodial. Furthermore that can be used as antimalarial drugs alternative or as a combination with antimalarials other.

Acknowledgements

We wish to acknowledgement to the Ministry of Research, Technology and Higher Education of the Republic Indonesia for the financial support for research in scheme Grant Competitive and Grant Post Graduate in the year of 2018.

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